

IN THE CLAIMS

1-23. (Canceled)

24. (Previously presented) A plant host cell or transgenic plant comprising a polynucleotide encoding an engineered zinc finger polypeptide and a target DNA sequence to which the zinc finger polypeptide binds.

25. (Currently amended) The plant host cell or transgenic plant of claim 24, wherein the zinc finger polypeptide has two or more zinc fingers and the zinc fingers structures of the formula

$X_{0-2} C X_{1-5} C X_{9-14} H X_{3-6} H/C$ (SEQ ID NO: 34)

wherein X is any amino acid and the numbers in subscript indicate possible numbers of residues represented by X.

26. (Previously presented) The plant host cell or transgenic plant of claim 25 wherein the zinc finger structures have a binding motif represented by:

$X^a C X_{2-4} C X_{2-3} F X^c X X X X L X X H X X X^b H$ – linker (SEQ ID NO: 22)

wherein each of X, X^a , X^b , X^c is any amino acid, the numbers in subscript indicate possible numbers of residues, and $X X X X L X X H X X$ between X^c and X^b are designated positions -1, 1, 2, 3, 4, 5, 6, 7, 8, and 9.

27. (Previously presented) The plant host cell or transgenic plant of claim 26 wherein X^a is E, K, T, S, Q, V, A or P, X^b is T or I, X^c is S or T, X_{2-4} is two amino acids, with the first of which being S, E, K, T, P, or R, and the second amino acid being E, and the linker is T-G-E-K (SEQ ID NO: 23) or T-G-E-K-P (SEQ ID NO: 24), and position 9 is Arg or Lys, and positions 1, 5, and 8 are hydrophobic amino acids and not Phe, Trp or Tyr.

28. (Previously presented) The plant host cell or transgenic plant of claim 26 wherein one or more of the zinc fingers binds to a target DNA triplet in accordance with the following:

- (a) if the 5' base in the triplet is G, then position 6 is Arg or position ++2 is Asp or position 6 is Arg and position 2 is Asp;
- (b) if the 5' base in the triplet is A, then position 6 is Gln or Glu and ++2 is not Asp;
- (c) if the 5' base in the triplet is T, then position 6 in is Ser or Thr and position ++2 is Asp or position 6 is a hydrophobic amino acid other than Ala;
- (d) if the 5' base in the triplet is C, then position 6 in may be any amino acid, provided that position ++2 is not Asp;
- (e) if the central base in the triplet is G, then position 3 is His;
- (f) if the central base in the triplet is A, then position 3 is Asn;
- (g) if the central base in the triplet is T, then position 3 is Ala, Ser, Ile, Leu, Thr or Val provided that if it is Ala, then one of the residues at -1 or 6 is a small residue;
- (h) if the central base in the triplet is 5-meC, then position 3 is Ala, Ser, Ile, Leu, Thr or Val provided that if it is Ala, then one of the residues at -1 or 6 is a small residue;
- (i) if the 3' base in the triplet is G, then position -1 is Arg;
- (j) if the 3' base in the triplet is A, then position -1 is Gln and position 2 is Ala;
- (k) if the 3' base in the triplet is T, then position -1 is Asn or position -1 is Gln and position 2 is Ser;
- (l) if the 3' base in the triplet is C, then position -1 is Asp and position 1 is Arg;

and,

when the central residue of a target triplet is C, the use of Asp at position 3 allows preferential binding to C over 5-meC; and,
wherein “++” residues are residues present in a C-terminal adjacent zinc finger, and when there is no C-terminal adjacent zinc finger, “++” interactions do not operate.

29. (Previously presented) The plant host cell or transgenic plant of claim 26 wherein there is an N-terminal zinc finger having a leader peptide MAEEKP (SEQ ID NO: 27) added thereto.

30. (Previously presented) The plant host cell or transgenic plant of claim 25 wherein one or more of the zinc fingers of the polypeptide comprises a mutated model zinc finger domain.

31. (Previously presented) The plant host cell or transgenic plant of claim 30 wherein the model zinc finger domain is a zinc finger from a protein selected the group consisting of Zif268, GLI, Tramtrack, or YY1.

32. (Previously presented) The plant host cell or transgenic plant of claim 25 wherein the zinc finger polypeptide has more than three zinc fingers.

33. (Previously presented) The plant host cell or transgenic plant of claim 32 wherein the zinc finger polypeptide has four, five, six, seven, eight or nine zinc fingers.

34. (Previously presented) The plant host cell or transgenic plant of claim 33 wherein the zinc finger polypeptide comprises zinc fingers 1-3 of TFIIIA, and three zinc fingers from Zif268, joined by zinc finger 4, including flanking sequences, of TFIIIA, acting as a linker.

35. (Previously presented) The plant host cell or transgenic plant of claim 24, wherein the target DNA sequence is operably linked to a coding sequence.

36. (Previously presented) The plant host cell or transgenic plant of claim 35, wherein transcription of the coding sequence is regulated by binding of the zinc finger polypeptide to the target DNA sequence.

37. (Previously presented) The plant host cell or transgenic plant of claim 24, wherein the target DNA sequence is part of an endogenous sequence.

38. (Previously presented) The plant host cell or transgenic plant of claim 24, wherein the target DNA sequence and coding sequence are heterologous to the cell.

39. (Previously presented) The plant host cell or transgenic plant of claim 24, wherein the zinc finger polypeptide is fused to a transcriptional activator domain.

40. (Previously presented) The plant host cell or transgenic plant of claim 34, wherein the zinc finger polypeptide is fused to a transcriptional activator domain.

41. (Previously presented) The plant host cell or transgenic plant of claim 40 wherein the transcriptional activator domain comprises VP16 transcriptional activator domain.

42. (Previously presented) The plant host cell or transgenic plant of claim 40 wherein the transcriptional activator domain comprises VP64 transcriptional activator domain.

43. (Previously presented) The transgenic plant host cell or transgenic plant of claim 25, wherein the zinc finger polypeptide is fused to a transcriptional repressor domain.

44. (Previously presented) The plant host cell or transgenic plant of claim 35, wherein the target DNA sequence is operably linked to a coding sequence.

45. (Previously presented) The plant host cell or transgenic plant of claim 40, wherein the target DNA sequence is operably linked to a coding sequence.

46. (Previously presented) The plant host cell or transgenic plant of claim 45 wherein the transcriptional activator domain comprises VP16 transcriptional activator domain.

47. (Previously presented) The plant host cell or transgenic plant of claim 45 wherein the transcriptional activator domain comprises VP64 transcriptional activator domain.

48 (Previously presented) The plant host cell of claim 25, wherein the zinc finger polypeptide is fused to a biological effector domain.

49. (Previously presented) The plant host cell or transgenic plant of claim 24 which is a transgenic plant.

50. (Previously presented) The plant host cell or transgenic plant of claim 24 which is a plant host cell.

51. (Previously presented) A method of regulating transcription in a plant cell from a DNA sequence comprising a target DNA operably linked to a coding sequence, which method comprises introducing an engineered zinc finger polypeptide into said plant cell which polypeptide binds to the target DNA and modulates transcription of the coding sequence.

52. (Currently amended) The method of claim 51, wherein the zinc finger polypeptide has two or more zinc fingers and the zinc fingers have structures of the formula

$X_{0-2} C X_{1-5} C X_{9-14} H X_{3-6} H/C$ (SEQ ID NO: 34)

wherein X is any amino acid and the numbers in subscript indicate possible numbers of residues represented by X.

53. (Previously presented) The method of claim 52 wherein the zinc finger structures have a binding motif represented by:

$X^a C X_{2-4} C X_{2-3} F X^c X X X X L X X H X X X^b H$ – linker (SEQ ID NO: 22)

wherein each of X, X^a , X^b , X^c is any amino acid, the numbers in subscript indicate possible numbers of residues, and $X X X X L X X H X X$ between X^c and X^b are designated positions -1, 1, 2, 3, 4, 5, 6, 7, 8, and 9.

54. (Previously presented) The method of claim 53 wherein X^a is E, K, T, S, Q, V, A or P, X^b is T or I, X^c is S or T, X_{2-4} is two amino acids, with the first of which being S, E, K, T, P, or R, and the second amino acid being E, and the linker is T-G-E-K (SEQ ID NO: 23) or T-G-E-K-P (SEQ ID NO: 24), and position 9 is Arg or Lys, and positions 1, 5, and 8 are hydrophobic amino acids and not Phe, Trp or Tyr.

55. (Previously presented) The method of claim 53 wherein one or more of the zinc fingers binds to a target DNA triplet in accordance with the following:

- (a) if the 5' base in the triplet is G, then position 6 is Arg or position ++2 is Asp or position 6 is Arg and position 2 is Asp;
- (b) if the 5' base in the triplet is A, then position 6 is Gln or Glu and ++2 is not Asp;
- (c) if the 5' base in the triplet is T, then position 6 in is Ser or Thr and position ++2 is Asp or position 6 is a hydrophobic amino acid other than Ala;
- (d) if the 5' base in the triplet is C, then position 6 in may be any amino acid, provided that position ++2 is not Asp;
- (e) if the central base in the triplet is G, then position 3 is His;
- (f) if the central base in the triplet is A, then position 3 is Asn;
- (g) if the central base in the triplet is T, then position 3 is Ala, Ser, Ile, Leu, Thr or Val provided that if it is Ala, then one of the residues at -1 or 6 is a small residue;
- (h) if the central base in the triplet is 5-meC, then position 3 is Ala, Ser, Ile, Leu, Thr or Val provided that if it is Ala, then one of the residues at -1 or 6 is a small residue;
- (i) if the 3' base in the triplet is G, then position -1 is Arg;
- (j) if the 3' base in the triplet is A, then position -1 is Gln and position 2 is Ala;
- (k) if the 3' base in the triplet is T, then position -1 is Asn or position -1 is Gln and position 2 is Ser;
- (l) if the 3' base in the triplet is C, then position -1 is Asp and position 1 is Arg;

and,

when the central residue of a target triplet is C, the use of Asp at position 3 allows preferential binding to C over 5-meC; and,
wherein “++” residues are residues present in a C-terminal adjacent zinc finger, and when there is no C-terminal adjacent zinc finger, “++” interactions do not operate.

56. (Previously presented) The method of claim 53 wherein there is an N-terminal zinc finger having a leader peptide MAEEKP (SEQ ID NO: 27) added thereto.

57. (Previously presented) The method of claim 52 wherein one or more of the zinc fingers of the polypeptide comprises a mutated model zinc finger domain.

58. (Previously presented) The method of claim 57 wherein the model zinc finger domain is a zinc finger from a protein selected from the group consisting of Zif268, GLI, Tramtrack, or YY1.

59. (Previously presented) The method of claim 52 wherein the zinc finger polypeptide has more than three zinc fingers.

60. (Previously presented) The method of claim 59 wherein the zinc finger polypeptide has four, five, six, seven, eight or nine zinc fingers.

61. (Previously presented) The method of claim 60 wherein the zinc finger polypeptide comprises zinc fingers 1-3 of TFIIIA, and three zinc fingers from Zif268, joined by zinc finger 4, including flanking sequences, of TFIIIA, acting as a linker.

62. (Previously presented) The method of claim 61, wherein the zinc finger polypeptide is fused to a transcriptional activator domain.

63. (Previously presented) The method of claim 62 wherein the transcriptional activator domain comprises VP16 transcriptional activator domain.

64. (Previously presented) The method of claim 62 wherein the transcriptional activator domain comprises VP64 transcriptional activator domain.

65. (Previously presented) The method according to claim 51 wherein the target DNA is part of an endogenous genomic sequence.

66. (Previously presented) The method according to claim 51 wherein the target DNA

and coding sequence are heterologous to the cell.

67. (Previously presented) The method according to claim 51 wherein the zinc finger polypeptide is fused to a biological effector domain.

68. (Previously presented) The method according to claim 67 wherein the zinc finger polypeptide is fused to a transcriptional activator domain.

69. (Previously presented) The method according to claim 67 wherein the zinc finger polypeptide is fused to a transcriptional repressor domain.

70. (Previously presented) The method according to claim 51 wherein the plant cell is part of a plant and the target sequence is part of a regulatory sequence to which the nucleotide sequence of interest is operably linked.--